THE BIOCHEMISTRY OF SPHINGOLIPIDS

By

GEORGE ELWOOD PHILLIPS

A.B., James Millikin, 1937

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE SCHOOL OF
THE UNIVERSITY OF ILLINOIS, 1944

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PART I

THE BIOCHEMISTRY OF SPHINGOLIPIDS

INTRODUCTION

Cerebrosides occur in greatest abundance in nervous tissue, but titrimetric procedure has indicated that they also exist in the blood (1). The availability of large amounts of blood plasma fractions[1] has made the following work possible. Galactose is the sole carbohydrate present in nervous tissue cerebrosides while glucose has been detected in spleen cerebrosides (2). These studies were undertaken in order to characterize the hexose and base present in blood cerebrosides. The location of the functional groups of sphingosine was also studied.

[1] The author is indebted to the Department of Physical Chemistry of Harvard University Medical School for the supply of human plasma protein fractions used in this investigation. The fractions were produced under contract with the Committee on Medical Research of the office of Scientific Research and Development from human blood provided by the American Red Cross.

EXPERIMENTAL

Blood plasma fractions were extracted successively with acetone, ether, hot alcohol and methanol-chloroform. The sphingolipids were obtained from the alcohol and methanol-chloroform extracts, and the cerebrosides isolated by reprecipitation from acetic acid. Nitrogen phosphorus, and cerebroside determinations were run on all fractions. Hydrolysis of the blood cerebrosides was carried out by a modification of the method of Klenk (3) and the sphingosine and carbohydrate were obtained. The preparation of triacetylsphingosine indicated that only sphingosine was present as no triacetyldihydrosphingosine could be detected. The hexose was converted to the aldonic acid which gave galacto-benzimidazol when condensed with *o*-phenylenediamine. No glucose could be detected. Studies were carried out in which galactose and glucose were treated in the same manner as the carbohydrate component of the cerebrosides. Glucose could be detected in the presence of galactose by fractionation of the aldo-benzimidazols when glucose comprised as little as one per cent of the mixture. It was concluded that no glucose was present in blood cerebrosides.

Sphingosine was isolated from nervous tissue cerebrosides and converted to the *N*-acyl- and triacyl derivatives. The *N*-acyl and triacyl

derivatives of dihydrosphingosine were also prepared. Neither *N*-acetyl nor *N*-benzoyldihydrosphingosine were oxidized when treated with periodic acid under a variety of conditions. Periodic acid is known to be a powerful and specific oxidant for 1, 2, glycols or 1, 2 amino alcohols. The oxidation of 1, 2 amino alcohols does not occur when the amino group is proteced by an acyl group. The failure of periodic acid to oxidize *N*-acyldihydrosphingosine derivatives proved that the hydroxy groups were not on adjacent carbon atoms, but were separated by a carbon atom containing the amino group. The unchanged starting materials were recovered in almost quantitative yields and converted to the tri-acyl derivatives. A sample of recovered *N*-benzoyldihydrosphingosine was treated with benzaldehyde in the presence of anhydrous zinc chloride. The resulting cyclic benzal derivative proved that the three functional groups were on adjacent carbon atoms. The structure of sphingosine was thus shown to be:

$$CH_3(CH_2)_{12}CH=CH-\underset{\displaystyle OH}{\underset{|}{CH}}-\underset{\displaystyle NH_2}{\underset{|}{CH}}-\underset{\displaystyle OH}{\underset{|}{CH_2}}$$

SUMMARY

Blood cerebrosides have been isolated and shown to contain only sphingosine as the base and galactose as the carbohydrate. Sphingosine has been shown to be 1, 3 dihydroxy-2-aminooctadecene-4.

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PART II

THE NUTRITIVE VALUE OF YEAST PROTEINS

INTRODUCTION

Torula yeasts have been suggested as a possible source of protein during periods of potential protein shortage. English workers (1) have developed strains which tolerate tropical climates and for this reason vast quantities of yeast may be grown in the West Indies, utilizing the cheap supply of carbohydrates. Conflicting results have been reported in studies of the value of yeast proteins (2, 3, 4). The present investigation was made to test the biological completeness of yeast protein by the rat growth method.

EXPERIMENTAL

Torula yeasts, brewers' yeast, and brewers' type yeast were fed to young albino rats. Casein was fed at a level of 12-18 per cent to the control animals and the experimental animals received equivalent amounts of yeast protein, allowance being made for 15 per cent of non-protein nitrogen in yeast. Brewers' yeast when fed at various levels supported as rapid a growth of rats as did casein fed at equivalent levels. Torula yeast did not support growth as efficiently as did casein. The difference was especially marked at low levels of feeding. Some toxic action was noted with Sprague-Dawley rats on brewers' yeast diets. No toxic action was shown with brewers' type yeast or Torula yeast. Yeast diets supplemented with methionine gave better growth than unsupplemented diets. Rats grew better on a diet in which the protein was furnished by a mixture of yeast and flour than on either yeast or flour alone. No acidosis was present in rats on yeast diets as shown by carbon dioxide combining power of the blood, and non-protein nitrogen values of the blood were not elevated.

SUMMARY

Yeast proteins are biologically complete, and the proteins of brewers' yeast and brewers' type yeast are equal in nutritive value to casein. Torula yeast, however, does not support growth as efficiently as casein. Methionine is the limiting amino acid of yeast proteins. Brewers' yeast and white

flour were shown to be supplementary. Rats on yeast diets did not suffer from acidosis or increased non-protein nitrogen in the blood.

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VITA

The author was born February 28, 1913, in Decatur, Illinois. He received his elementary and secondary education in the public schools of Decatur, Illinois. He attended James Millikin University from which he received his Bachelor of Arts degree in June, 1937. He attended Illinois State Normal University during the summer of 1937. The author was then employed by the Decatur Sanitary District where for the following three years he held the position of chemist. On April 30, 1938, he married Joy Burcham. He entered the graduate school of the University of Illinois in September 1940. From 1941 until 1943 he served as an analyst in the Department of Agronomy. In August, 1943, he received an appointment as special research assistant under a grant from the Nutrition Foundation.

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